REMARKS

Applicant thanks the Examiner for her consideration of applicant's December 23, 2008 Response.

Interview Summary

Applicant thanks the Examiner for the courtesies extended during the June 25, 2009 telephonic interview. Examiner Strzelecka, Jim Haley, Kenichi Hartman and Matthew Ashby participated in the interview and discussed claims 45-50 and 57 and the Declaration of Matthew Ashby filed on December 23, 2008. During the interview, applicant and the Examiner discussed various exhibits that applicant had sent to the Examiner via e-mail. An agreement was not reached.

Sequence Rules Compliance

The Examiner contends that the sequence of the 1392R primer referred to in Figure 13 is essential for the best mode embodiment, and that its sequence needs to be included in the sequence listing.

The sequence of the 1392R primer is not a part of the claimed invention, and is not essential for the best mode embodiment. The design of a PCR primer is well-known in the art. See arguments regarding 35 U.S.C. §112, best mode, infra. As such, the sequence of the 1392R primer does not need to be included in the sequence listing.

The Claim Amendments

Claims 45, 47-50 and 58-69 are pending.

Applicant has amended claim 45 to read: "A culture-independent method of determining the abundance of an environmental parameter in an environmental sample comprising the steps of: a. providing a first plurality of environmental samples at least some of which samples contain the environmental parameter; b. isolating a plurality of genomic DNAs from each of the samples provided in step a; c. isolating a plurality of 16S rRNA gene segments

from each plurality of genomic DNAs isolated in step b; d. determining the abundance of each of said 16S rRNA gene segments in each plurality of 16S rRNA gene segments isolated in step c; e. determining the abundance of the environmental parameter in each of the samples provided in step a; f. correlating the abundance of each 16S rRNA gene segment determined in step d with the abundance of the environmental parameter determined in step e; g. selecting at least one 16S rRNA gene segment whose abundance correlates to the abundance of said environmental parameter, as determined in step f; h. providing an environmental sample set of at least one environmental sample; i. isolating a plurality of genomic DNAs from each environmental sample of the environmental sample set provided in step h; j. determining the abundance of said 16S rRNA gene segment selected in step g in each plurality of genomic DNAs isolated in step i; and k. inferring the abundance of the environmental parameter in each environmental sample of the environmental sample set provided in step h based upon the abundance of said 16S rRNA gene determined in step j in each environmental sample of the environmental sample set provided in step h." Support for amended claim 45 may be found throughout the specification as originally filed, for example, in the abstract; at page 1, lines 8-16; at page 6, line 5 to page 7, line 27; at page 13, line 8 to page 14, line 20; at page 17, lines 8-25; at page 24, line 8 to page 30, line 1; in Example 3; and in Figures 6, 9 and 11.

Applicant has amended claims 47-49 and 50 to improve their form and to fix antecedent basis in light of amended claim 45.

Claims 58-69 are new.

Support for claims 58-59 and 67-68 may be found, for example, at page 27, line 25 to page 28, line 20.

Support for claim 60 may be found, for example, in the abstract; at page 1, lines 8-16; at page 6, line 5 to page 7, line 27; at page 13, line 8 to page 14, line 20; at page 17, lines 8-25; at page 24, line 8 to page 30, line 1; in Example 3; and in Figures 6, 9 and 11.

Claims 61-63 are based on claims 47-49. Support for these claims may be found, for example, at page 28, line 24 to page 29, line 21.

Claims 64 and 66 are based on claim 50. Support for these claims may be found, for example, at page 7, lines 20-27; page 14, lines 18-20; and page 30, line 3 to page 31, line 21.

Support for claim 69 may be found in the application as originally filed, for example, at page 17, lines 8-12; page 25 lines, 3-13; and page 25, line 28 to page 26, line 45.

New claims 58-68 are readable upon the elected species.

New Claim 69 is not readable upon the elected species. Applicant requests that, upon allowance of the generic claims from which claim 69 depends, the Examiner consider claim 69 and allow it, as provided by 37 C.F.R. § 1.141.

Applicant has canceled claims 46 and 57. Applicant's cancellation of any subject matter is without prejudice or waiver of his right to pursue that subject matter in an application claiming priority or benefit herefrom.

Rejections

35 U.S.C. §112, first paragraph – Best mode

Claims 45-50 and 57 stand rejected under 35 U.S.C. §112, first paragraph, as allegedly failing to comply with the best mode requirement. The Examiner contends that there is evidence of concealment of the best mode because the amplification of the 16S rRNA is a prerequisite to the successful performance of the claimed invention, and the lack of a sequence for the 1392R primer as shown in Figure 13 precludes others from even trying to repeat applicant's steps. Applicant traverses.

There was no intention on the part of applicant to conceal the best mode of the invention. The amplification of a gene from isolated genomic DNA was well known in the art at the priority date of the application. As such, the lack of a sequence for the 1392R primer does not preclude others from carrying out applicant's steps. Further, there are no suggestions in the application that said primer is applicant's best mode. Indeed, many primers can be used to effect the desired amplification.

Applicant requests that the Examiner reconsider and withdraw the best mode rejection.

35 U.S.C. §112, first paragraph – Enablement

The Examiner argues that applicant's arguments filed December 23, 2008 are not persuasive. The Examiner has maintained the rejection of claims 45-50 and 57 under 35 U.S.C. §112, first paragraph, as allegedly failing to comply with the enablement requirement. The Examiner argues that the specification has no working examples of how to obtain a correlation between an abundance of a nucleic acid marker sequence and an abundance of an environmental parameter, and further contends that the guidance provided by the specification amounts to a mere invitation for the skilled worker to try and follow the disclosed instructions to make and use the claimed invention. Further, the Examiner contends that the art to which the invention is directed is unpredictable and that it is not possible to reliably determine the abundance of different organisms in environmental samples and, therefore, to correlate such abundances to environmental parameters.

The Examiner, in response to applicant's arguments presented on December 23, 2008, has made the following further contentions:

a) Particular primers, restriction enzymes, ligation adapters, and the obtaining of 16S rRNA tags described in the specification, upon which the method relies, are not in the claims.

b) The results presented in the Declaration by Matthew Ashby, also presented on December 23, 2008 ("the Declaration") were obtained at an unspecified time. The disclosure needs to be enabling at the time of the filing of the application.

c) The claims are very broad and can encompass, e.g., colon with gut bacteria, tree bark from a forest, sample of sewage water, etc., each with corresponding challenges.

Applicant traverses and will now address these further contentions in light of the amended claims.

Applicant has canceled Claims 46 and 57 without prejudice and the rejection is moot with respect to those claims. Claim 45 (and claims 47-50 that depend therefrom), as amended, is directed to a method of identifying a 16S rRNA gene segment based on the correlation of its abundance to the abundance of an environmental parameter in a plurality of environmental samples, which gene segment is then used to infer the abundance of the parameter in an envirenmental sample set.

With regard to contention a), the claimed methods do not rely on the particular primers, restriction enzymes or ligation adapters. However, the identification of a 16S rRNA gene segment and its use in inferring the abundance of environmental parameters are recited in claim 45 as amended (and in claims 47-50 that depend therefrom).

With regard to contention b), applicant note that the submission of the Declaration was not meant to suggest that enabling disclosure is lacking from the application as originally filed. Enabling disclosure for the claimed invention can be found throughout the application as originally filed. See supra. Rather, the Declaration serves merely to illustrate and to confirm the enabling disclosure that was present in the application as originally filed. Therefore, the claimed invention is enabled regardless of the timing of when the results in the Declaration were obtained.

With regard to contention c), applicant respectfully disagrees that "environment sample" is overly broad. The method of isolating genomic DNA from a wide variety of sources was well known in the art at the time of invention. See, e.g., page 17, lines 16-25 of the specification. Further, after genomic DNA is isolated from an environmental sample of any kind, the source of said genomic DNA does not affect the execution of the subsequent steps of the method in any way. How the invention is practiced is completely identical regardless of whether the DNA is originally isolated from a colon, a tree bark, a sample of sewage water, or any other sample.

The Examiner, in addition, has maintained the contention that the invention cannot be practiced as claimed because it is not possible to reliably determine the abundance of different

organisms in environmental samples. The Examiner refers to Witzengerode et al. (FEMS Microbiol. Rev. 21: 213-229, 1997; "Witzengerode") and Colbert et al. (Appl. Env. Microbiol., 59: 2056-2063; "Colbert") as supposed support for this contention. The Examiner points to Witzengerode to support her contention that PCR amplification of environmental samples is prone to error and is unreliable. Colbert recites that, following the introduction of salicylate, the number of microbial cells increased, then remained constant. The Examiner interprets this report to mean that making a correlation between the presence of such substances and the number of bacteria would be impossible. Applicant traverses.

As previously demonstrated in the December 23, 2008 Response to Office Action (see page 13), the alleged sources of error in the PCR amplification of environmental samples recited in Witzengerode can be effectively mitigated by the use of appropriate techniques and conditions known in the art at the time of the invention. Further, the variation in copy number of the rRNA operons in microbes (pointed to by Witzengerode and the Examiner) is irrelevant to the claimed process. The claimed method looks to relative differences in the number of copies of specific gene sequences in one sample vs. other samples for identifying gene segments whose abundance correlates with environmental parameters. As for Colbert, applicant previously demonstrated that Colbert does not affect the enablement of the claimed invention, because the Declaration shows that the Examiner's assertion is mistaken, and because Colbert's observations that cells only increased or remained constant is an exception rather than a general phenomenon.

The Examiner, in the instant Office Action, responds to the above arguments and further contends that:

d) Applicant argued limitations not in the claims because the claims do not require determining a relative number of bacteria between samples.

e) Applicant argued limitations not in the claims because the claims do not require samples to be collected over a certain period of time.

Applicant traverses and will now address the further contentions d) and e) in light of the amended claims.

As for contention d), claim 45 (and claims 47-50 that depend therefrom), as amended, is directed to a method of identifying a 16S rRNA gene segment based on the correlation of its abundance to the abundance of an environmental parameter across a plurality of samples. As such, Claim 45 includes steps for determining correlations between the abundance of the gene sequences and environmental parameters (e.g., see step f). Therefore, applicant is not arguing limitations not in the claims.

As for contention e), applicant respectfully suggests that the Examiner has misunderstood applicant's argument. Applicant previously stated that "the observation made in Colbert is not relevant to the claimed invention because the experiments in Colbert were only conducted over a short period of time" (see the December 23, 2008 Response to Office Action, page 13). The above statement was not meant to present or imply a particular feature of the claimed invention (i.e., that samples should be collected over a certain period of time). Rather, it supports applicant's assertion that Colbert's observations of cells only increasing or remaining constant would be an exception rather than a general phenomenon. Therefore, applicant is not arguing limitations not in the claims.

Further, the correlation and selection steps in amended claim 45 (i.e. steps f and g) provide additional bases for the irrelevance of Colbert in the context of the claimed invention. Any 16S rRNA gene segment that increases and remains constant (as the cells observed by Colbert behaved) would simply be one among many 16S rRNA gene segments whose abundance do not correlate with the environmental parameter and, therefore, not selected as the indicator. That some (or even many) 16S rRNA gene segments would not correlate with an environmental parameter (like those referred to in Colbert) would not prevent the claimed invention from being practiced.

For the above reasons, the claimed invention is fully enabled in the application as filed. Applicant requests that the Examiner reconsider and withdraw the enablement rejection.

35 U.S.C. §102(b) – Anticipation

Claims 45-50 and 57 stand rejected under 35 U.S.C. §102(b) as being allegedly anticipated by Wikstrom et al. ("Wikstrom"). The Examiner contends that Wikstrom recites a

culture-independent method of determining the abundance of an environmental parameter (i.e., PAHs) by determining the abundance of a nucleic acid marker sequence (i.e., catechol 2,3-doixygenase gene), wherein the abundance of the nucleic acid marker sequence correlates to the abundance of the environmental parameter. Applicant traverses in light of the amended claims.

Applicant has canceled claims 46 and 57, thus render this rejection moot as to those claims. Claim 45 (and claims 47-50 that are dependent therefrom), as amended, are directed to a method for identifying a 16S rRNA gene segment based on the correlation of its abundance to the abundance of an environmental parameter across a plurality of samples, which gene segment is then used infer the abundance of the environmental parameter in a set of environmental samples. Wikstrom does not recite such a method. Wikstrom, by the Examiner's own admission, differs from the claimed invention, in that catechol 2,3-doixygenase genes were chosen for the assaying of samples based on the known function of the 2,3-doixygenase protein in metabolizing PAHs.

For the above reasons, Wikstrom does not anticipate the claimed invention, and applicant requests that the Examiner reconsider and withdraw the anticipation rejection.

35 U.S.C. §103(a) – Obviousness

Claims 47-49 stand rejected under 35 U.S.C. §103(a) as being allegedly obvious over Wikstrom as evidenced by Clarke et al. ("Clarke"). The Examiner contends that since Wikstrom recites a correlation between the abundance of the catechol-2,3 dioxygenase gene and the PAH concentration in the sample, and it was also well known in the art at the time of the invention how to determine a correlation coefficient between two variables, as shown by Clarke, it would have been obvious to one of ordinary skill in the art at the time of the invention to represent the data of Wikstrom in a form of correlation coefficients as customary in the art. Applicant traverses.

As discussed above, claim 45, from which claims 47-49 depend, differs from Wikstrom in a number of important and patentable ways beyond the representation of data in the form of correlation coefficients. Unlike Wikstrom, claim 45 is directed to a method for identifying a gene based on the correlation of its abundance to the abundance of an

environmental parameter across a plurality of samples, which gene is then used infer the abundance of the environmental parameter in an environmental sample set. Further, and again unlike Wikstrom, claim 45 is directed to choosing a gene based on the correlation of its abundance to the abundance of an environmental parameter, rather than based on a previously known function of the gene. Clarke is brought by the Examiner to merely contend that the determination of correlation coefficients was well known in the art at the time of the invention. Therefore, even with the knowledge of determining correlation coefficients, as allegedly shown by Clarke, one of ordinary skill in the art would not have made the claimed invention.

Applicant requests that the Examiner reconsider and withdraw the obviousness rejection.

CONCLUSION

Applicant requests consideration of the amended claims in view of the foregoing remarks and that the Examiner allows pending claims 45, 47-50 and 58-69.

Should the Examiner feel that a telephone conference with applicant's representative would be helpful, she is invited to telephone the undersigned at any time.

Respectfully submitted,

/JAMES F. HALEY, JR./

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